Role of microRNAs in breast cancer

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MicroRNAs (miRNAs) are a class of post-transcriptional gene regulators with critical functions in normal cellular processes as well as disease processes. They are small molecules with 18~23 nucleotides in length. Since their early discovery in 1993, a large number of miRNAs have been characterized and analyzed to understand their pivotal role and their impact in a myriad of biological processes. Substantial research on miRNA highlights the involvement of these tiny RNAs in the etiopathogenesis of a variety of human diseases such as cancer, neuro-degenerative disorders, diabetes, cardiac hypertrophy and respiratory diseases. In this review, we update on recent advances of the emerging role of miRNAs in breast cancer and their clinical implications.

Introduction

RNA was originally known to be a messenger, relaying information from DNA of genes to the cytoplasm for protein synthesis. However, the noncoding and the non-messenger part of the RNA molecule are what today's scientists are fascinated by. MicroRNAs (miRNAs) are a class of endogenous small RNAs that have revealed a new level of gene regulation in the cell. They also form vital components of the central dogma machinery and are found widely in the genome of high eukaryotic cells. It is believed that miRNA genes constitute about 1–2% of the known genes in eukaryotes.[1,2] They have been shown to regulate key processes of gene activation and suppression. A single miRNA can coordinate multiple pathways involving the regulation of multiple genes. On the contrary, several different miRNAs can together control a single miRNA target. This multifarious role leads to the complexity of gene regulation, thus mediating complex network of eukaryotic cell function,[3] including hematopoietic cell differentiation, apoptosis, cell proliferation and organ development.[4]

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MicroRNAs as Master Gene Regulators

It is well known now that miRNAs regulate gene expression through a posttranscriptional mechanism, including mRNA degradation and translation repression. For example, miRNAs accelerate mRNA turnover by allowing the removal of the poly(A) tail from messages to which they are partially complementary.[5,6] Deadenylation and the consequent loss of poly(A)-binding protein trigger 5' decapping, thereby promoting exonucleolytic digestion from the 5' end.[7,8] A number of studies suggest that miRNA-mediated gene expression is also through translation repression. For example, studies have revealed a shift of targeted mRNAs to translationally active polysomes of lower mass, which indicates impaired initiation.[9] Other studies have suggested that miRNAs can only inhibit translation, initiated by a cap-dependent mechanism.[9,10] Moreover, recent findings implicate that miRNAs cause an m^7 G cap-dependent hindrance to the recruitment of 80S ribosomes to mRNA.[11-13] Thus, the entire translational repression mechanism can be explained on the basis of the cap binding affinity of the miRNA-binding protein Ago, where the cap is concealed such that it cannot bind to the initiation factor eIF4E.[14]

In fact, there are a number of proteins which help to mediate translational repression and mRNA degradation. In particular, some of these proteins are integral components of the RNA-induced silencing complex (RISC) that delivers those small RNAs to complementary sites within mRNA.[15,16] There is overwhelming evidence suggesting that miRNAs exert their silencing function usually by interactions with the 3'-untranslated region (3'-UTR) of a target gene through imperfect base-pairing. This unique feature implies that a single miRNA can have multiple targets and cellular factors associated with RISC complex may influence miRNA targeting specificity. One consequence of the interactions is that miRNAs could regulate a large number of protein-coding genes. Since many of these miRNA targets are involved in various signaling pathways, their impact on gene expression can be significantly amplified. Because of this feature, miRNAs can function as master gene regulators, impacting a variety of cellular pathways important to normal cellular functions as well as cancer development and progression.

Breast Cancer Subtypes and MicroRNAs

Breast cancer is a complex disease characterized by heterogeneity of genetic alterations. According to the American Cancer Society, excluding the cancers of skin, breast cancer is the most common cancer among women, accounting for nearly one in three cancers diagnosed in US women. Technological advancements in the last decade have helped the researches to understand this complex disease more thoroughly. Based on gene expression profile and the phenotype, the breast cancer can be divided into six major subtypes. The six major subtypes are luminal A, luminal B, tumor enriched with human epidermal growth factor receptor 2 (Her2), basal-like, normal-like and claudin-low subtype.[17-20] Among them, luminal A is the most common subtype characterized by the expression of estrogen receptor (ER), progesterone receptor (PR), Bcl-2 and absence of Her2. It accounts for 50–60% of the total breast cancer cases.[18,19] The luminal B subtype is characterized by the expression of ER, PR and absence of Her2. They can be differentiated from luminal A subtype on the basis of high Ki67 staining which indicates higher proliferation rate.[21] Both luminal A and luminal B subtypes are treated with adjuvant endocrine therapy such as tamoxifen and aromatase inhibitors, both of which target the ER signaling. These two tumor subtypes are associated with more favorable prognosis. Her2 positive subtype constitutes 15–20% of all the breast cancer. It is characterized by the high expression of the Her2 gene and high proliferation rate. Her2 positive tumors are treated using trastuzumab, an antibody against Her2. Although the survival rate has improved in the last decade the overall prognosis still remains poor. In contrast to other tumor subtypes, basal-like breast cancer expresses none of the three markers (ER, PR and Her2), accounting for 10–20% of all breast carcinomas, and is often associated with a poor prognosis. Although the treatment includes the conventional chemotherapeutic approach, promising strategies are being developed to treat basal-like breast cancer, such as poly-ADP ribose polymerase-1 inhibitors.[20,22]

Normal-like subtype is the rarest form of breast cancer subtype which accounts for only 5–10% of all breast carcinomas. Although poorly characterized this subtype has been shown to express ER, Her2 and PR and has a clinical outcome between basal like and luminal A subtype. It has a strong basal epithelial gene expression, are triple negative but lack expression for CK5 and EGFR.[20] Some researchers have also expressed their concern over the real existence of this breast cancer subtype as they could not confirm it by microarray approach. Their doubt is based on the possible contamination of cancerous cells with their surrounding normal tissue.[23] Claudin-low breast cancer subtype is triple negative in nature characterized by low expression of claudin-3, 4, 7, ocludin and E-cadherin with cancer stem like features. Claudin is a tight junction protein whereas E-cadherin is a calcium dependent cell-cell adhesion glycoprotein. This subtype has relatively low frequency in the range of 12–14% and a poor long-term prognosis.[24]

Of considerable interest, a unique miRNA expression pattern can be associated with certain subtype. For example, miR-21, miR-210 and miR-221 are significantly overexpressed in triple-negative (ER, PR and Her2) breast cancer, whereas miR-10b, miR-145, miR-205 and miR-122a are significantly underexpressed in these cancer types. Furthermore, miR-21, miR-210 and miR-221 correlate with worse patient disease-free and overall survival, and hence they play a significant role in triple-negative primary breast cancers.[22] In a separate study, comprehensive miRNA expression profiling was performed in normal basal and luminal breast epithelium cells isolated from plastic surgery. Out of 664 miRNAs analyzed, 116 are expressed in normal breast epithelium, among which eight miRNAs are significantly upregulated in normal basal cells, including let7c, miR-125b, miR-126, miR-127-3p, miR-143, miR-145, miR-146-5p and miR-199a-3p, whereas miR-200c and miR-429 are mostly luminal. Furthermore, miR-126, miR-127, miR-143, miR-145 and miR199 are significantly high in myoepithelioma as compared with luminal and basal-like subtypes. Levels of miR-200c and miR-429 are lower in malignant myoepithelioma than those in luminal and basal-like breast cancer.[25] Lowrey et al. used miRNA profiling in 29 early stage breast cancer specimens to determine the status of ER, PR and Her2 based on miRNA signature which could further help determine breast cancer subtype and prognosis. The artificial network analysis identified miR-342, miR-299, miR-217, miR-190, miR-135b, miR-218 as ER specific, miR-520 g, miR-377, miR-527-518a, miR-520f-520c as PR specific and miR-520d, miR-181c, miR-302c, miR-376b and miR-30e as Her2 specific. Further analysis revealed that miR-342 expression was highest in ER and Her2 positive luminal B tumors and lowest in triple-negative tumors whereas miR-520 g expression was downregulated in ER and PR-positive tumors. Importantly, this miRNA signature not only helps determine breast cancer phenotype but also indicate therapeutic targets and markers for prognosis.[26]

Dysregulation of MicroRNAs in Human Breast Cancer

Several studies have been conducted to identify the miRNAs that are differentially expressed and regulate breast cancer initiation and progression in different breast cancer subtypes. miRNA analysis in 76 breast cancer and 10 normal breast samples led to the identification of 29 miRNAs whose expression is significantly dysregulated with the most significantly dysregulated miRNAs being miR-125b, miR-145, miR-21 and miR-155; 15 of them could correctly predict the nature of the sample analyzed (i.e., tumor or normal breast tissue).[27] In order to identify miRNAs capable of regulating transition from ductal carcinoma in situ to invasive ductal carcinoma, 94 biopsies were analyzed and of interest, a nine-miRNA signature was found to be specific for invasiveness and five miRNAs were associated with time to metastasis and overall survival. Specifically, let-7d, miR-210 and miR-221 were downregulated in the ductal carcinoma in situ and upregulated in the invasive transition.[28] In another study, 100 breast cancer patients and 89 healthy patients were recruited for miRNA genotyping and expression profiling using peripheral blood to detect and identify characteristic patterns. miR-499, miR-146a and miR-196a-2 in postmenopausal patients and miR-196a-2 in premenopausal patients may distinguish breast cancer

patients from healthy individuals.[29] In an attempt to identify the specific miRNAs that are co-expressed in the serum and tissue of breast cancer (BC) patients, a genome-wide miRNA analysis by next-generation sequencing was conducted in 13 BC patients and 10 healthy controls. miRNAs found to be co-upregulated include miR-103, miR-23a, miR-29a, miR-222, miR-23b, miR-24 and miR-25. Among these, miR-222 is significantly increased in the serum of BC patients which may serve as a valuable biomarker for differentiating BC patients from normal individuals.[30]

One of the major reasons for the mortality in breast cancer is metastasis.[31] Metastasis is a complex process during which a primary solid tumor invades the adjacent tissue and then spreads to the neighboring as well as the distant parts of the body.[32] Epithelial to mesenchymal transition (EMT) is thought to be an important component at the early stages of metastasis which is followed by extravasation, colonization of metastatic tissues and finally process of differentiation which is similar to mesenchymal to epithelial transition (MET).[33,34] Several recent studies reported the involvement of miRNAs in the process of metastasis where some miRNAs are remarkably downregulated or eliminated, whereas other miRNAs are significantly accumulated in metastatic breast cancer specimen.[35] The miRNAs which promote the metastasis in breast cancer include miR-9,[36] miR-10b,[37,38] miR-21,[39-45] miR-29a,[46] miR-155[47] and miR-373/520 family.[48] For example, miR-9 contributes to the increased cell motility and invasion ability in breast cancer cells by targeting E-cadherin, activating the β-catenin pathway and increasing the vascular endothelial growth factor (VEGF) levels.[36] Tristetraprolin is a target for miR-29a, and it regulates EMT and metastasis in breast cancer.[46] CD44 is a cell surface molecule involved in multiple physiological cell functions including differentiation, proliferation, migration, angiogenesis and cell survival. Targeting of CD44 by miR-373/520 can enhance the migration and invasion ability in vitro and in vivo. Clinical metastasis samples also showed an inverse correlation between miR-373 and CD44 expression.[49] miR-10 family and miR-21 are very important miRNAs contributing to tumor metastasis, and we will discuss more details about them later.

On the other hand, miRNAs as metastasis suppressors in breast cancer include miR-7,[50-52] miR-17/20,[53,54] miR-22,[55-57] miR-30,[58,59] miR-31,[60-62] miR-126,[63-68] miR-145,[69-72] miR-146,[73,74] miR-193b,[75] miR-205,[76,77] miR-206,[78-80] miR-335,[32,81] miR-448,[82] miR-661[83,84] and let-7.[85-87] We select a few miRNA targets to illustrate the important role of these genes in metastasis. The metastasis suppressive function of miR-7 has been attributed to its ability to target epidermal growth factor receptor (EGFR) which regulates important cellular processes like proliferation and differentiation, and is frequently overexpressed in different cancers including breast cancer.[52] miR-7 can also inhibit p21-activated kinase 1 expression which is a signaling kinase involved in multiple cancers. Overexpression of miR-7 in highly invasive and tumorigenic breast cancer cell line, MDA-MB-231, results in suppression of invasion ability, motility, anchorage independence and tumorigenesis.[88] Although miR-17 may function as an oncogenic miRNA in other types of cancer, miR-17/20 has been reported to target cyclin D1, which is a cell cycle regulatory subunit promoting the G_1-S phase transition. Increased cyclin D1 expression has been reported in ~50% of human breast cancers and has an inverse correlation with miR-17/20. Overexpression of miR-17/20 in breast cancer cell lines suppresses cell proliferation and arrests the cell cycle at the G_1 phase.[54,89] The metastasis suppressor action of this miRNA cluster has been reported in breast cancer where the cell conditioned medium from non-invasive breast cancer cell MCF-7 can inhibit invasion and migration ability of highly invasive MDA-MB-231 cell line. This effect is likely mediated through direct inhibition of IL-8 and inhibition of cytokeratin 8 through cyclin D1.[53] miR-22 was reported to inhibit tumor growth and metastasis in vivo using a mouse model of breast carcinoma. Upon overexpression, miR-22 can target the genes involved in senescence which includes CDK6, SIRT1 and Sp1, inducing senescence in addition to decreased cell motility and invasion in vitro.[55] A separate study showed that miR-22 can also target estrogen receptor α (ERα) and has inhibitory effect on ERα dependent breast cancer cell proliferation.[57] Work in our lab showed that miR-30 can directly target Ubc9, an E2-conjugating enzyme involved in sumoylation. Since Ubc9 has significant role in cell growth and cancer development, its inhibition by overexpressing miR-30 suppresses cell growth. A similar correlation is also seen in breast tumor specimens where Ubc9 expression is about 6-fold higher than in the matched normal breast tissue.[58] Moreover, enforced expression of miR-30 can inhibit self-renewal capacity of BT-ICs through Ubc9 and induces apoptosis by targeting ITGB3 expression. Particularly, BT-IC xenograft overexpressing miR-30 can reduce tumorigenesis and lung metastasis in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mouse model.[59] One of the well-known tumor suppressor miRNAs is miR-145, which can target multiple gene like IRS-1, mucin-1, c-myc, JAM-A and fascin.[69-72] More details about miR-145 will be discussed later. miR-146 is capable of targeting various important suppressors of tumorigenesis and metastasis. When ectopically expressed in metastatic breast cancer cell line MDA-MB-231, miR-146 negatively regulates NFκB via downregulating interleukin receptor associated kinase and TNF associated factor 6.[74] miR-146 can be upregulated by breast cancer metastasis suppressor 1 and can directly downregulate EGFR, thus significantly reducing the pulmonary metastasis in the MDA-MB-231 mouse model.[73]

MicroRNAs and Breast Cancer Stem Cells

One of the major obstacles toward treatment of breast cancer is the fraction of the cells that are able to initiate new tumor growth and are often resistant to standard chemotherapeutic drugs.[90] These are the breast cancer stem cells (BCSCs) which are often characterized based on their ability to initiate tumors in immunocompromised or syngeneic mice, self-renewal capacity as measured by tumor formation in secondary mice and also the ability to differentiate into the non-self-renewing cells, which constitute the tumor bulk.[91] BCSCs are commonly identified using the characteristic CD44^{+}/CD24^{-/low} marker profile or Aldefluor assays.[92,93] It has been proposed that miRNAs contribute to the

maintenance of tumor-initiating or stem cell properties and characteristic miRNA expression signature can predict the various features of breast cancer including metastasis.[94] miRNA expression profiling studies in the breast cancer stem cells indicates differential expression pattern as compared with normal mammary stem and/or progenitor cells. The study performed by Yu et al. used chemotherapy in mice undergoing passaging of breast cancer SKBR3 cells to selectively enrich for self-renewing BCSCs. The tumors raised are enriched with a high percentage of CD44^{+}/CD24^{-/low} cells with high ability to form mammospheres in vitro and a high rate of forming tumors capable of serial transplantation as xenografts. Importantly, the miRNAs that were significantly downregulated in the BCSC-enriched cell population includes let-7 along with miR-16, miR-107, miR-128 and miR-20b, as compared with the parental and differentiated cells. Furthermore, they provide evidence that let-7 is also reduced in BCSC from clinical cancer specimens and reduced let-7 expression is required to maintain mammospheres. Increased let-7 level inhibits tumor formation in NOD/SCID mice and the tumors are less likely to metastasize.[95]

Shimono et al. reported that 37 miRNAs are differentially expressed in CD44^{+}/CD24^{-/low} BCSCs as compared with non-tumorigenic cancer cells. In particular, three clusters, miR-200c-141, miR-200b-200a-429 and miR-183-96-182, are significantly downregulated. Importantly, miR-200c inhibits the clonogenicity of breast cancer cells and suppresses the cancer cell growth and induces differentiation. Moreover, miR-200c strongly suppresses the normal mammary outgrowth in vivo and tumor formation driven by human BCSCs. Their work indicates that both normal and BCSCs share common molecular mechanisms regulating stem cell functions.[92] Furthermore, Chang et al. showed that p53 directly binds to the miR-200c promoter and transactivates it. Loss of p53 leads to a decreased level of miR-200c and an increase in the expression of EMT and stemness markers, leading to the development of a high tumor grade.[96]

Han et al. cultured and isolated ALDH1^{+} and CD44^{+}/CD24^{-/low} cells from breast cancer MCF-7 parental cells to evaluate the role of miR-21 in regulating the BCSC-like cell biological features, especially EMT. They found that HIF-1α and miR-21 are upregulated in the BCSC-like cells and reduction in miR-21 expression by antagomir leads to reversal of EMT, downexpression of HIF-1α, as well as suppression of invasion and migration. This indicates that miR-21 regulates EMT transition in BCSC as well as overexpression of HIF-1α.[97]

Transforming growth factor and its family has a significant regulatory contribution in normal as well as CSCs. Wang et al. demonstrated that upon exposure to TGF-β there is an increase in the population of BCSCs and this effect is mediated via miR-181. The target of miR-181, ataxia telangiectasia mutated (ATM), is reduced in mammospheres upon TGF-β treatment. When miR-181 is upregulated or ATM is depleted in breast cancer cells, there is an induction in sphere formation. These results led to the conclusion that TGF-β pathway regulates the BCSCs property by interfering with the tumor suppressor ATM through miR-181 which itself is upregulated by TGF-β at the post-transcriptional level.[98]

Of interest, Hwang et al. isolated a distinct subpopulation of breast cancer stem cells which were PROCR^{+}/ESA^{+} instead of usual CD44^{+}/CD24^{-/low} cells and performed miRNA expression profiling which showed miR-495 as the most highly upregulated miRNA in these cells. This miRNA is also upregulated in CD44^{+}/CD24^{-/low} population, highlighting its significance in maintaining BCSC properties. E-cadherin is a direct target of miR-495 expression and upon overexpression, it promotes cell invasion and inhibits REDD1 expression to increase cell proliferation through a posttranscriptional mechanism.[99]

Key MicroRNAs That Play an Important Role in Breast Cancer

Although a plethora of miRNAs have been reported to be implicated in breast cancer initiation, progression and/or metastasis, we will discuss three groups of miRNAs which can act as oncogene or tumor suppressor or play a role in breast cancer therapy resistance.

Oncogenic miRNAs. Oncogenic miRNAs are frequently upregulated in cancer and they act by repressing the expression of tumor suppressor gene/s mainly involved in apoptosis, cell proliferation, cell migration and invasion and metastasis.[100] Following are the few important miRNAs and miRNA families that have been extensively studied and established as oncomirs in breast cancer.

miR-10 family. The miR-10 family constitutes of miR-10a and miR-10b and found to be retained within the Hox cluster of developmental regulators. Since they regulate Hox transcripts, it is likely that this miRNA family may play pivotal roles during the development process.[101] miR-10 family has been reported to be dysregulated in various cancers including glioblastoma,[102] colon cancer,[103] acute myeloid leukemia,[104] melanoma,[105] pancreatic cancer[106] and hepatocellular carcinoma.[107] In case of breast cancer, miR-10 family is reported to be involved both in the development and metastasis through miR-10a and miR-10b, respectively.[105]

miR-10b is highly overexpressed in metastatic breast cancer cells and induces cell migration and invasion in murine xenograft model of breast cancer via targeting HOXD10 gene.[37] A recent report indicated that E-cadherin is a potential target for miR-10b and expression level of miR-10b has a positive correlation with tumor size, pathological grading, clinical staging, lymph node metastasis, Her2-positivity and tumor proliferation, but is negatively correlated with ER[+], PR[+] and E-cadherin levels. miR-10b is induced by transcription factor twist which is able to enhance invasion ability and metastasis in breast cancer cells and animal model by targeting HOXD10 in addition to E-cadherin and Tiam1.[37,38,108] miR-10b suppression of HOXD10 results in elevated level of pro-metastatic gene, RHOC, which is a member of Rho GTPase family, known to regulate actin dynamics and hence cell shape and motility.[109] miR-10b inhibits Tiam1 (T lymphoma invasion and metastasis)-mediated Rac activation and controls cell-cell adhesion and EMT through E-cadherin and suppresses the ability of breast cancer cells to invade and migrate.[110] Hence, suppression of E-cadherin by miR-10b may

modulate breast cancer metastasis. Thus, miR-10b could be a biomarker of advanced progression and breast cancer metastasis.[108] However, caution should be taken in this regard, because a study conducted in patients with primary breast cancer reveals no significant association between miR-10b levels and metastasis or prognosis.[111]

miR-21. miR-21 is one of the important miRNAs associated with cell migration and invasion of breast cancer cells and hence contributes to tumor progression and metastasis.[112,113] Aberrant expression of miR-21 was first reported by Chan et al. showing markedly elevated levels in human glioblastoma tumor tissues and established glioblastoma cell lines compared with non-neoplastic fetal and adult brain tissues.[114] During the same year, Iorio et al. reported that miR-21, along with other miRNAs including miR-125b, miR-145 and miR-155 are also aberrantly expressed in human breast cancer, as shown by microarray and then confirmed by northern blot analyses.[115] In consistent with these reports, our work with real-time RT-PCR array analysis showed that miR-21 is highly overexpressed in breast tumors compared with the matched normal breast tissues.[116] Through two-dimensional differentiation in-gel electrophoresis of tumors treated with anti-miR-21, we identified the tumor suppressor tropomyosin 1 (TPM1) as a potential target of miR-21.[43] Subsequently, we identified two additional direct miR-21 targets, programmed cell death 4 (PDCD4) and maspin, both of which have been implicated in invasion and metastasis.[44] The role of maspin (mammary serine protease inhibitor) in breast cancer as invasion and metastasis suppressor is well established because it can suppress invasion ability, angiogenesis and induce apoptosis.[117-119] Expression of miR-21 is negatively correlated with expression of PTEN in breast cancer and also correlates with advanced stage and metastasis and poor survival.[120,121] It has been observed in breast cancer that transforming growth factor-β (TGF-β) can upregulate the expression of miR-21. Elevated miR-21 expression is associated with aggressive disease status which includes high tumor grade, negative hormone receptor status and ductal carcinoma. Samples collected from 344 patients diagnosed with primary breast cancer indicate that despite no value for prognosis, a high level of miR-21 is associated with poor disease-free survival in early stage patients.[122]

miR-17-92 cluster. miR-17-92 is a polycistron and is located in a region of DNA that is amplified in human B-cell lymphomas.[123] It comprises six mature miRNAs including miR-18b, miR-19b, miR-20a, miR-92, miR-93 and miR-106.[124] This miRNA cluster was found to be amplified in lymphomas.[125] It was also reported that miR-17-92 cluster is markedly overexpressed in lung cancers, especially with small-cell lung cancer.[126] As discussed earlier, this miRNA cluster may play a metastasis suppression role.[53] However, miR-17-5p was shown to be highly expressed in invasive MDA-MB-231 breast cancer cells but not in non-invasive MCF-7 breast cancer cells. Ectopic expression of this miRNA in MCF-7 cells can lead to more invasive and migratory phenotypes by targeting HBP1/β-catenin pathway. Similarly, downregulation of miR-17-5p suppresses the migration and invasion of MDA-MB-231 cells in vitro.[127]

Tumor Suppressor MicroRNAs

let-7 family. Reinhart et al. identified let-7 in *Caenorhabditis elegans* as an essential factor for cell type determination during embryogenesis.[128] The let-7 family is comprised of 10 members identified in humans, including let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98 and miR-202. Normal physiological functions of let-7 include muscle formation, cell adhesion and regulation of gene expression and development. let-7 biogenesis is regulated by Lin28 which have been shown to act as a post-transcriptional repressor.[128,129] let-7 expression is downregulated in numerous types of cancer, including lung cancer,[130] gastric cancer,[131] colon cancer[132] and Burkitt lymphoma.[133] In breast cancer it is lost at an early stage of disease progression.[134] Yu et al. investigated the role of let-7 in breast cancer stem cells and used breast tumor initiating cells (BT-IC) and non-BT-IC from primary breast cancer for their self-renewal, differentiation and tumorigenicity. They found that let-7 family miRNAs were markedly reduced in BT-IC and increased with differentiation. Upon overexpression, let-7 reduces proliferation, mammosphere formation of BT-IC in vitro and tumor formation and metastasis in NOD/SCID mice. Targets for let-7 include H-RAS and HMGA2, suggesting that let-7 regulates BT-IC stem cell-like properties by silencing multiple targets.[85]

miR-200 family. miR-200 family consists of five members which include miR-200a, miR-200b, miR-200c, miR-141 and miR-429. miR-200 family are the suppressors of EMT, which is in part mediated through the regulation of E-cadherin transcriptional repressors ZEB1 and ZEB2 (also known as SIP1).[135-137] miR-200 family was found to be lost in invasive breast cancer cell lines with mesenchymal phenotypes and also in regions of metaplastic breast cancer specimens lacking E-cadherin. Ectopic expression of the miR-200 family significantly increases E-cadherin expression and alters cell morphology to an epithelial phenotype.[136,137] In this regard, miR-200c regulates breast cancer cell migration, stress fiber formation, migration, invasion and elongation and thus, metastasis by targeting FHOD1 and PPM1F which are direct regulators of the actin cytoskeleton.[138] Downregulation of miR-200c is also associated with drug resistance in human breast cancer.[139]

However, the role of miR-200 family in breast cancer metastasis still remains controversial. Although accumulating evidence suggests that miR-200 family regulates the E-cadherin expression and EMT transition, this does not necessarily predict the metastatic potential. miR-200 family has been shown to regulate PLCG1, BMI1, TGF-β2, FAP-1, ZEB and Suz12, hence acting as tumors suppressor.[137,140-148] On the other hand, this miRNA family regulates transcription factor ZEB (Zinc-finger enhancer binding) which is activator of EMT and overexpression of this miRNA in mouse breast cancer cell lines unexpectedly leads to macroscopic metastasis.[149] Korpal et al. also strengthen this notion in clinical and experimental models of breast cancer metastasis. Overexpression of miR-200 family in weakly metastatic 4TO7 cells promotes metastatic colonization in part through inhibition of Sec23a mediated secretion of IGFBP4 and Tinag1, both of which act as metastasis suppressors.[150] Evidently,

further investigation is needed to define its role in metastasis. However, cellular content may also play a role in this aspect.

miR-205. Like miR-200 family, miR-205 is also significantly downregulated in cells that had undergone EMT in response to TGF-β and in the triple-negative primary breast cancers.[137,151] In situ hybridization (ISH) analysis of formalin-fixed and paraffin-embedded specimens representing normal and tumor tissue from more than 100 patient cases revealed that expression of miR-205 is restricted to basal epithelium of normal mammary ducts and lobules as compared with reduced or lost expression in matching tumor specimens.[152] We also reported that miR-205 is significantly underexpressed in breast tumor compared with the matched normal breast tissue.[76] Ectopic expression of miR-205 in breast cancer cells inhibits invasion, proliferation and anchorage independent growth. This effect is in part through direct targeting of Her3 and VEGF-A.[76] A separate study by a different group reported that miR-205 can also target Her3 and interferes with the PI3K/Akt survival pathway in addition to improving responsiveness to tyrosine kinase inhibitor drugs like gefitinib and lapatinib.[77] p53-induced miR-205 can act as a tumor suppressor in triple negative breast cancer and its re-expression can strongly reduce cell proliferation, cell cycle progression and clonogenic potential in vitro and inhibit tumor growth in vivo. The experimentally validated targets for miR-205 include E2F1 and LAMC1 which are regulators of cell cycle progression and cell adhesion, proliferation and migration, respectively.[153]

miR-145. Iorio et al. reported that miR-145 is significantly downregulated in breast cancer specimen compared with normal breast tissue, as demonstrated by microarray and northern blot analyses. Like miR-205, miR-145 is significantly underexpressed in breast tumor compared with the matched normal breast tissue. Due to early manifestation of altered miR-145 expression in breast cancer, this miRNA may have a clinical diagnostic potential as a novel biomarker for early cancer detection.[152] Spizzo et al. demonstrated the tumor suppression function of miR-145 in breast cancer cell lines wherein miR-145 has pro-apoptotic potential which is dependent on TP53 activation. Furthermore, TP53 activation can, in turn, stimulate miR-145 expression promoting a positive regulatory death loop. In addition, miR-145 can directly target estrogen receptor-α (ER-α) protein expression through direct interaction and promotes apoptosis in both ER-α positive and wild type TP53-expressing breast cancer cells.[154] Our lab showed that miR-145 can directly target and silence a well-known oncogene c-Myc, which regulates cell growth and proliferation leading to inhibition of tumor cell growth both in vitro and in vivo. Also, expression of miR-145 is induced transcriptionally by p53 through interaction of p53 response element in the promoter region of miR-145.[70] miR-145 also plays a role in suppression of metastasis in breast cancer. For example, while miR-145 has no significant effect on cell growth in metastatic breast cancer cell lines it can inhibit invasion ability of these cells, partly due to the silencing of the metastasis gene mucin 1 (MUC1). This silencing of MUC1 further causes a reduction of β-catenin as well as the cadherin 11, which has oncogenic potential.[71] In addition, miR-145 has been shown to target cell-cell adhesion protein, JAM-A and fascin in breast cancer cell lines; suppression

of these targets can drastically decrease breast cancer cell motility.[72] Kim et al. evaluated the therapeutic potency of miR-145 against breast cancer and found that adenoviral construct of miR-145 (Ad-miR-145) can suppress cell growth and motility in both the in vitro and in vivo systems. Furthermore, a treatment of Ad-miR-145 combined with 5-FU showed a significant anti-tumor effect, as compared with 5-FU alone.[155] A study conducted in 106 cases of normal and breast cancer tissues revealed that miR-145 was significantly downregulated in breast tumors. miR-145 can target and regulate N-RAS and VEGF-A at the post-transcriptional level, leading to inhibition of tumor angiogenesis, tumor growth and invasion as N-RAS and VEGF-A are required for these phenotypes.[156]

MicroRNAs Involved in Resistance to Breast Cancer Therapy

One of the major drawbacks and obstacles toward breast cancer therapy is chemoresistance. This resistance is largely attributed to genetic and epigenetic modifications. The key genes that are involved in breast cancer drug resistance include multidrug resistance 1 (MDR1) which codes for P-glycoprotein, multidrug resistance-associated protein (MRPs), breast cancer resistant protein (BCRP), glutathione S-transferase π (GSTπ) and DNA repair genes such as O^6-methylguanine DNA methyltransferase (MGMT).[157,158] The first three genes belong to ATP-binding cassette (ABC)-superfamily multidrug efflux pumps. Their normal physiological function is to pump out the toxic metabolites and xenobiotics to protect the cells.[157] The human GSTπ is a phase II detoxification enzyme and has been reported to contribute to chemoresistance in many cancers by mediating the efflux of chemotherapeutic drugs especially cisplatin from the cells.[159] MGMT is a DNA repair enzyme and often found to be methylated in brain tumors. It confers chemoresistance by targeting alkylating agents as demonstrated in several cell lines and xenograft models.[160,161] Accumulating evidence suggests that chemoresistance and miRNAs are closely related as these miRNAs can target and modulate the key genes involved in breast cancer therapy resistance. For example, MDR1 gene has been shown to be directly regulated by miR-451 and miR-298 in doxorubicin resistant and sensitive MCF-7 and MDA-MB-231 cells. Upon overexpression, these miRNAs can downregulate P-glycoprotein, thereby increasing nuclear localization and cytotoxicity of doxorubicin in resistant breast cancer cells.[162,163] Similarly MRP-1 has been reported to be overexpressed in VP-16 resistant MCF-7 cell line as compared with parent cell line. Also, miR-326 expression was shown to be downregulated in MCF-7/VP cells as determined by miRNA microarray, but upon overexpression of miR-326, these cells become sensitive to VP-16 and doxorubicin.[164] Other miRNAs regulating the MRP-1 gene includes miR-7 and miR-345; MRP-2 is shown to be regulated by miR-489.[162,165] Other important miRNAs that are involved in breast cancer therapy resistance are summarized in **Table 1** along with their drugs and potential targets.

Another major hurdle toward breast cancer chemotherapy is the involvement of breast cancer stem cells (BCSCs). It is
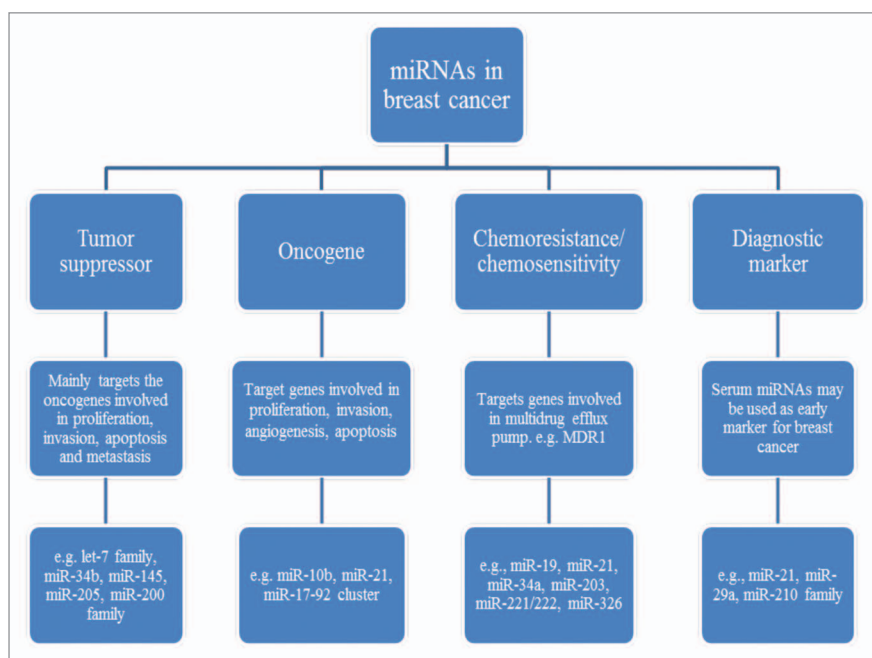
Table 1. miRNAs involved in breast cancer treatment resistance and chemosensitivity along with their target molecules

miRNA	Cell lines used or clinical specimen	Drug used	Potential targets	Effect	References
miR-221/222	MCF-7 cells	Tamoxifen, fulvestrant	p27Kip1, ER α, β-catenin	Chemoresistance	168–170
miR-328	MCF-7/MX100 cells	mitoxantrone	BCRP/ABCG2	Chemosensitivity	171
miR-326	MCF-7, MCF-7/VP	VP-16 and doxorubicin	MRP-1	Chemoresistance	172
miR-128a	MCF-7, MCF-7aro	letrozole	TGFβRI	Chemoresistance	173
miR-345 and miR-7	MCF-7	Cisplatin	MRP-1	Chemosensitivity	174
miR-155	Breast tumor specimen, MDA-MB-157, MDA-MB-435 and HS578T	Paclitaxol, VP-16 and doxorubicin	FOXO3a	Chemoresistance	175
miR-125b	MDA-MB-435 MDA-MB-231 MDA-MB-436 MCF7, SKBr3 and BT474	Paclitaxol	Bak1	Chemoresistance	176
miR-342	MCF-7/HER2, MCF-7/HER2Δ16, Breast Tumor samples	Tamoxifen	NR4A2, MAGED2, SEMA3D	Chemosensitivity	177
miR-34a	MCF-7, MDA-MB-231	Docetaxel	BCL-2 and CCND1	Chemoresistance	178
miR-21	Breast Tumor samples, MCF-7/ADR	Trastuzumab, doxorubicin	PTEN	Chemoresistance	179, 180
miR-19	MCF-7, MDR cell lines, subcutaneous breast cancer animal models	Taxol, VP-16 and mitoxantrone	PTEN	Chemoresistance	181
miR-128	Breast tumor–initiating cells	Doxorubicin	Bmi-1 and ABCC5	Chemosensitivity	182
miR-200c	Breast Tumor samples, MCF-7/ADR	Doxorubicin	MDR1	Chemosensitivity	183
miR-203	Human breast cancer tissues, MCF-7	Cisplatin	SOCS3	Chemoresistance	184
miR-298	MDA-MB-231	Doxorubicin	MDR1	Chemoresistance	185

believed that cancer stem cells also contribute to breast cancer development, progression and chemoresistance. These BCSCs are often characterized by the expression of CD44 but absence or low expression of CD24.[166] In particular, they can also be distinguished from non-BCSCs in tumor by increased expression of ABC transporter proteins which in turn are responsible for protecting cells from chemotherapy through efflux pumping mechanisms.[167] Hence, BCSCs, although they comprise a small proportion of breast cancer population, could be a major contributor for chemoresistance. In this regard, miRNAs play a very significant role as they not only can regulate the formation of cancer stem cells but also they directly impact expression of resistance genes.

Perspective

Breast cancer is the best studied cancer in terms of pathobiology, subtypes and treatment, and thus, the role of miRNAs in breast cancer is well characterized. Here, we have discussed three groups of miRNAs, however, due to multiple targeting feature, miRNAs can often regulate several pathways. More importantly, miRNAs exert their silencing function through a complex process which may depend on cellular content, revealing tissue or cell type-specific phenotypes. A better understanding of the miRNA-guided network will open new windows for diagnostics (**Fig. 1**) and therapy of breast cancer. In this regard, miRNAs could be potential biomarkers for

Figure 1. Functional characterization of different miRNAs in breast cancer.

predicting a response to systemic therapy and prognosis in clinical settings. For instance, targeting specific miRNAs of the drug-resistant network is promising in overcoming drug resistance in breast cancer. In particular, combinations of different anticancer agents with miRNA reagents such as anti-miR-21 may provide more effective therapeutic approaches. Therefore, the clinical applications of miRNA studies may rely on successful identification of miRNA signatures as cancer biomarkers and development of miRNA-based targeted therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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